Description: The pSV40-CLuc Control Plasmid is a mammalian expression vector that encodes the secreted luciferase from the Ostracod *Cypridina noctiluca* (1) as a reporter, under the control of the constitutive SV40 promoter. *Cypridina* Luciferase (CLuc) is a 62 kDa protein with a native signal peptidase at the N-terminus that allows it to be secreted from mammalian cells (1) so that CLuc activity can be detected in the culture medium. There is a multiple cloning site (MCS) upstream of the SV40 promoter.

Source: Isolated from an *E. coli* strain NEB10β by standard DNA purification methods.

Supplied in: 10 mM Tris-HCl (pH 7.5 @ 25°C), 1 mM EDTA.

Advantages:
- Multiple samples can be obtained from the same transfected cells (i.e., before and after experimental treatments or at multiple time points).
- 90–95% of CLuc activity is found in the cell culture medium, with the remaining 5-10% detectable in cell lysates (Figure 1). This allows flexibility when assaying CLuc along with other co-transfected reporters.
- The activity of CLuc is high and the CLuc assay is sensitive enough to detect very small amounts of CLuc enzyme activity.
- CLuc does not use the same substrate as other marine luciferases (e.g. *Renilla*, *Gaussia*). Therefore, it is possible to assay both CLuc and GLuc independently in cell culture medium from cells expressing both reporters.
- The pSV40-CLuc Control Plasmid can be transfected into cells using any standard transfection protocol.

Applications:
- The pSV40-CLuc Control Plasmid can be used as a control for assessing the efficiency of transfection in mammalian cells. Plasmids containing other constitutive promoter elements are also available (see Companion Products Sold Separately). CLuc can be used as a stand alone reporter or in conjunction with other compatible reporters such as *Gaussia* luciferase (GLuc) (2). CLuc and GLuc are ideally suited for co-expression as both are secreted and highly active enzymes providing ease of use and sensitivity.

Features of pSV40-CLuc Control Plasmid:
- Polylinker MCS: 1–51
- SV40 promoter: 51–246
- CLuc ORF: 291–1952
- Start codon of CLuc: 291–293
- Stop codon: 1950–1952
- Signal peptide: 291–344
- SV40 poly-A site: 1967–2188
- SV40 enhancer: 2195–2441
- Bacterial replication ori (pMB1): 3347–2759
- Amp resistance: 4378–3518

Figure 1: Cypridina Luciferase (CLuc) activity obtained from different CLuc plasmids. HeLa cell supernatants (20 µl) and lysates (5 µl) were assayed with the BioLux CLuc Assay Kit (NEB #E3309). HeLa cells were seeded in 12-well plates and transfected with 50 ng of CLuc-expressing plasmid per well. At 24 hr post-transfection, supernatants were collected and cell lysed in 100 µl well using Luciferase Cell Lysis Buffer (NEB #E3309). The CLuc activity was measured in a Mithras LB940 (Berthold Technologies) luminometer set to 50 µl of injection, 2 seconds of delay and 2 seconds of integration.

Goals of pSV40-CLuc Control Plasmid:
- pSV40-CLuc can be transfected into cells using any standard transfection protocol.
- pSV40-CLuc can be co-transfected with other compatible reporters such as *Gaussia* luciferase (GLuc) (2).
- The pSV40-CLuc Control Plasmid can be used as a control for assessing the efficiency of transfection in mammalian cells.
- pSV40-CLuc can be used as a stand alone reporter or in conjunction with other compatible reporters such as *Gaussia* luciferase (GLuc) (2).
- CLuc and GLuc are ideally suited for co-expression as both are secreted and highly active enzymes providing ease of use and sensitivity.
Recommended sequencing primers for pSV40-CLuc

**CLuc:**
- **Forward primer (23-mer) (not available from NEB)**
  5´-GAGTTCAAGAAAGAATGCTACAT-3´ (1888–1910)
- **Reverse primer (24-mer) (not available from NEB)**
  5´-GTAAGGACAGTCCTGGCAATGAAC-3´ (360–337)

**Frequently Asked Questions:**

Where can I find the sequence of this plasmid?
The sequences of all the plasmids sold by NEB are available online at www.neb.com.

Can I make a stable cell line with pSV40-CLuc?
No. The pSV40-CLuc Control Plasmid does not contain a Neo<sup>+</sup> marker for selection in mammalian cells.

Can I transfect this plasmid into mammalian cells?
Yes. In general, for transfection one will need to use plasmid DNA from CsCl prep or Qiagen Maxi Prep.

How do I assay for CLuc expression?
Please refer to the BioLux<sup>®</sup> CLuc Assay Kit (NEB #E3309).

Can I use assay kits designed for other reporters (Gaussia, Renilla & Firefly luciferases) to assay CLuc activity?
No. **Cypridina** Luciferase catalyzes the light reaction using a different substrate that is not the same as those for **Gaussia**, **Renilla** & Firefly luciferases. Therefore, the CLuc activity can only be assayed by using the BioLux CLuc Assay Kit (NEB #E3309).

Is there another secreted reporter that can be used with CLuc?
Yes. **Cypridina** and **Gaussia** are both secreted luciferases, which produce high bioluminescent signal intensity. They oxidize different substrates that do not cross-react with each other. Therefore, **Cypridina** and **Gaussia** are an ideal duo for co-transfecting mammalian cells (2). Refer to the BioLux **Gaussia** Luciferase (GLuc) Assay Kits and GLuc expression vectors for more information.

**References:**


**Companion Products Sold Separately:**

- **BioLux Cypridina Luciferase Assay Kit**
  - #E3309S 100 assays
  - #E3309L 1,000 assays
- **pCLuc-Basic 2 Vector**
  - #N0317S 20 µg
- **Luciferase Cell Lysis Buffer**
  - B3321S 25 ml
- **BioLux Gaussia Luciferase Assay Kit**
  - #E3308S 100 assays
  - #E3308L 1,000 assays
- **BioLux Gaussia Luciferase Flex Assay Kit**
  - #E3300S 100 assays
  - #E3300L 1,000 assays
- **pCMV-GLuc 2 Control Plasmid**
  - #N8081S 20 µg
- **pGLuc Basic 2 Vector**
  - #N8082S 20 µg
- **pTK-GLuc Vector**
  - #N8084S 20 µg
- **pGLuc Mini-TK 2 Vector**
  - #N8086S 20 µg

This product is intended for research purposes only. This product is not intended to be used for therapeutic or diagnostic purposes in humans or animals.

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**References:**


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For use of the BioLux Cypridina Luciferase Assay Kit, or associated assay reagents, in human diagnosis and measurement in relation to human health, contact busdev@neb.com.